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Review

Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence

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ABSTRACT

Over the last decade, numerous studies have investigated the role of oxygen in setting thermal tolerance in aquatic animals, and there has been particular focus on arthropods. Arthropods comprise one of the most species-rich taxonomic groups on Earth, and display great diversity in the modes of ventilation, circulation, blood oxygen transport, with representatives living both in water (mainly crustaceans) and on land (mainly insects). The oxygen and capacity limitation of thermal tolerance (OCLTT) hypothesis proposes that the temperature dependent performance curve of animals is shaped by the capacity for oxygen delivery in relation to oxygen demand. If correct, oxygen limitation could provide a mechanistic framework to understand and predict both current and future impacts of rapidly changing climate.

In arthropods, most studies testing the OCLTT hypothesis have considered tolerance to thermal extremes. These studies likely operate from the philosophical viewpoint that if the model can predict these critical thermal limits, then it is more likely to also explain loss of performance at less extreme, non-lethal temperatures, for which much less data is available. Nevertheless, the extent to which lethal temperatures are influenced by limitations in oxygen supply remains unresolved. Here we critically evaluate the support and universal applicability for oxygen limitation being involved in lethal temperatures in crustaceans and insects.

The relatively few studies investigating the OCLTT hypothesis at low temperature do not support a universal role for oxygen in setting the lower thermal limits in arthropods. With respect to upper thermal limits, the evidence supporting OCLTT is stronger for species relying on underwater gas exchange, while the support for OCLTT in air-breathers is weak. Overall, strongest support was found for increased anaerobic metabolism close to thermal maxima. In contrast, there was only mixed support for the prediction that aerobic scope decreases near critical temperatures, a key feature of the OCLTT hypothesis. In air-breathers, only severe hypoxia (<2 kPa) affected heat tolerance. The discrepancies for heat tolerance between aquatic and terrestrial organisms can to some extent be reconciled by differences in the capacity to increase oxygen transport. As air-breathing arthropods are unlikely to become oxygen limited under normoxia (especially at rest), the oxygen limitation component in OCLTT does not seem to provide sufficient information to explain lethal temperatures. Nevertheless, many animals may simultaneously face hypoxia and thermal extremes and the combination of these potential stressors is particularly relevant for aquatic organisms where hypoxia (and hyperoxia) is more prevalent. In conclusion, whether taxa show oxygen limitation at thermal extremes may be contingent on their capacity to regulate oxygen uptake, which in turn is linked to their respiratory medium (air vs. water).

Fruitful directions for future research include testing multiple predictions of OCLTT in the same species. Additionally, we call for greater research efforts towards studying the role of oxygen in thermal limitation of animal performance at less extreme, sub-lethal temperatures, necessitating studies over longer timescales and evaluating whether oxygen becomes limiting for animals to meet energetic demands associated with feeding, digestion and locomotion.

Abbreviations: MMR, maximum metabolic rate — the maximum rate at which oxygen can be consumed at a given temperature; SMR, standard metabolic rate — the minimum rate of oxygen consumption needed to sustain life in resting, post-absorptive organisms at a given temperature; MMR − SMR, absolute aerobic scope (AAS); MMR/SMR, relative aerobic scope, sometimes also referred to as factorial aerobic scope; OCLTT, hypothesis, oxygen and capacity limited thermal tolerance hypothesis; T_{opt}, optimal temperature — the temperature where an organism can achieve maximum aerobic scope (MMR − SMR); T_{pejus}, pejus temperature — the temperature beyond which aerobic metabolism declines rapidly and hypoxemia sets in; T_{crit}, critical temperature — the temperature beyond which aerobic metabolism is no longer sufficient to cover energy demand and anaerobic metabolism sets in; CT_{max}, critical thermal maximum — an empirical endpoint of heat tolerance found in heating trials where the temperature is ramped up, indicating the temperature at which the animal becomes moribund and can no longer escape the adverse temperatures; CT_{min}, critical thermal minimum — as for CT_{max}, but reflecting the cold tolerance.

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Contents

1. Oxygen supply and demand in thermal biology

Temperature is a key determinant of species geographic ranges (Addo-Bediako et al., 2000; Root et al., 2003; Calosi et al., 2010; Sunday et al., 2010; Kellermann et al., 2012a,b; Overgaard et al., 2014) and current range expansions and contractions are tightly linked to global climate change (Rosenzweig et al., 2008; Sunday et al., 2012). A thorough understanding of the physiological mechanisms underpinning the thermal tolerance of intact living organisms is therefore essential to predict the impacts of current and future rises in global temperatures (Chown, 2012; Huey et al., 2012). Extreme temperatures directly impact a myriad of organismal functions at all levels of biological organization, with effects ranging from protein denaturation, membrane instability, cell disruption and organ failure. At less extreme levels, altered temperature changes performance, including exercise ability, energy assimilation or reproductive capacities at the whole-animal level (Cossins and Bowler, 1987; Schmidt-Nielsen, 1997; Feder and Hofmann, 1999; Pörtner, 2002; Angilletta, 2009). In the long term, such thermal effects on performance can impact population dynamics (reviewed in e.g., Angilletta, 2009; Chown and Terblanche, 2007). Despite the obvious multifaceted effects of temperature on living organisms, recent studies have focused on insufficient aerobic metabolism as the weakest link in thermal tolerance of ectothermic animals (e.g., Frederich and Pörtner, 2000; Pörtner, 2001, 2002, 2006, 2010; Mark et al., 2002; Klok et al., 2004; Davenport and Davenport, 2007; Pörtner and Knust, 2007; Stevens et al., 2010; Verberk and Bilton, 2011, 2013, 2015; Overgaard et al., 2012; Clark et al., 2013; Ern et al., 2014; Fobian et al., 2014; Norin et al., 2014; Wang et al., 2014; Bjelde et al., 2015; Boardman and Terblanche, 2015; Deutsch et al., 2015; Koopman et al., 2016; Lefevre et al., in press). Given the ~15 fold greater energy gains associated with aerobic metabolism compared to anaerobic metabolism, animals depend on a continuous and adequate flux of oxygen from their surroundings to their metabolizing tissue to maintain energy status. In ectotherms, standard metabolic rate (SMR) increases with temperature, reflecting elevated energetic costs associated with maintenance, including for example enhanced protein turnover and increased ion pumping (Hochachka and Somero, 2002). However, the maximum rate of oxygen uptake (MMR) does not always increase with temperature and may plateau or even decrease at high temperatures as shown for several fish (e.g., Fry and Hart, 1948). The difference between SMR and MMR represents excess capacity for aerobic metabolism and hence energy generation, reflecting the ability of organisms to meet energy demands associated with activity, feeding, growth and reproduction, etc. Through its different effects on MMR and SMR, temperature influences both the absolute aerobic scope (MMR − SMR) and relative aerobic scope (MMR/SMR) (Fig. 1).

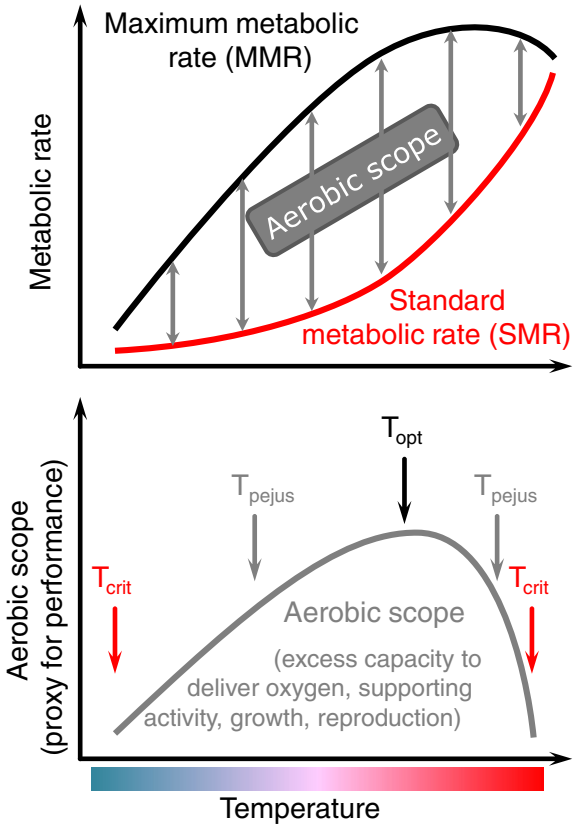

Fig. 1. Thermal dependency of standard metabolic rate (SMR) and maximum metabolic rate (MMR), as well as the difference between both, the aerobic scope. Aerobic scope is maximized at "intermediate" temperatures (T_{opt}) because of the different temperature dependence of SMR and MME (top panel). Aerobic scope is used as a proxy for animal fitness, as it governs aerobic energy available for activity, feeding, growth and reproduction (bottom panel). Aerobic scope declines at T_{pejus} and can no longer be sustained beyond T_{crit}.

2. The oxygen and capacity limited thermal tolerance hypothesis (OCLTT)

The oxygen and capacity limited thermal tolerance (OCLTT) hypothesis has been proposed to explain thermal performance curves of animals by integrating various mechanisms from most levels of biological organization into a larger context of animal evolution (Pörtner, 2010; Storch et al., 2014). These mechanisms relate to oxygen delivery (involving both ventilatory oxygen uptake and cardiovascular oxygen transport) and oxygen utilization at the level of mitochondria to maintain aerobic metabolism. One of the central aspects of the OCLTT hypothesis is the thermal dependence of aerobic scope (Fig. 1), which is argued to decline as temperatures deviate from optimum (T_{opt}), where aerobic scope is maximal. Interestingly, several species of fish prefer body temperatures that coincide with maximal performance (e.g., maximal aerobic scope, growth or swimming performance) (Fry

and Hart, 1948; Brett, 1971; Pörtner and Knust, 2007), although this generality has recently been contested (Clark et al., 2013). According to the OCLTT hypothesis, beyond threshold temperatures (T_{pejus}), aerobic scope is reduced because a mismatch develops between the oxygen supply capacity and oxygen demand. The resulting energy deficiency causes performance to decline, which in the long-term constrains fitness and hence defines the thermal niche of the animal. Under acute exposure to temperatures beyond critical thermal limits (T_{crit}), aerobic metabolism can no longer support energy demand and the animal succumbs, although death may be postponed by compensatory anaerobic metabolism (Pörtner, 2001, 2002, 2010). The idea of oxygen-limited thermal tolerance has historical predecessors (Winterstein, 1905; Fry and Hart, 1948; Brett, 1971), but the OCLTT hypothesis differs by stressing the long term effects of capacity limitations, and by incorporating the role of the mitochondria. As ATP production depends not only on oxygen availability but also on functional mitochondria capable of using the oxygen, the OCLTT hypothesis extends beyond oxygen supply capacity. Indeed, a part of the OCLTT hypothesis deals with how to maintain mitochondrial function and the costs involved in doing so. In relation to cold limits, mitochondrial failure has been emphasized, resulting in falling ATP production rates, which then has consequences for maintaining oxygen delivery as energy demands posed by ventilation and circulation efforts can no longer be met. In relation to heat limits, insufficient capacity for oxygen delivery at the systemic level (i.e., the ventilatory and circulatory systems of the whole organism) is argued to first cause the onset of thermal limitation (e.g., Storch et al., 2014), subsequently resulting in tissue hypoxia and falling ATP production rates. Whether or not oxygen delivery is insufficient depends on the oxygen supply capacity in relation to oxygen (energy) expenditure. Time considerations are important here since dealing with acute thermal challenges will be fundamentally different from dealing with chronic heat stress (e.g., Cossins and Bowler, 1987; Schulte et al., 2011). Animals may increase oxygen delivery (e.g., increase ventilation rates and heart rates) and decrease oxygen demand by altering energy allocation to growth and reproduction or by entering a hypometabolic state, although such responses cannot suffice on the long term without impairing fitness.

The OCLTT hypothesis has been argued to be generally applicable across animal taxa, building on the premise that thermal limitation at the whole organism level should be sought at the highest complexity level (Pörtner, 2006; Storch et al., 2014). However, recent studies mainly focussing on acute responses to temperature have questioned the generality of the OCLTT hypothesis for aquatic (Clark et al., 2013; Ern et al., 2014; Norin et al., 2014; Wang et al., 2014; Lefevre et al., in press) and terrestrial, air-breathing animals (Klok et al., 2004; Stevens et al., 2010; Overgaard et al., 2012; Verberk and Bilton, 2013; Fobian et al., 2014; Boardman and Terblanche, 2015). Hence, it remains uncertain whether the OCLTT is indeed a "unifying" model to understand thermal tolerance of extant animals. Alternatively, insufficient oxygen delivery in relation to demand could just be one of several physiological processes that compromise performance near thermal limits (i.e., loss of growth and reproduction near thermal limits could in theory also result from failure of protein function, ion homeostasis and/or membrane function before tissue hypoxia sets in) (see Clark et al., 2013; Ern et al., 2015; Schulte, 2015). In other words, here we raise the question of whether there is any *a priori* reason to expect insufficient oxygen delivery to be the primary driver of thermal tolerance?

Over the last decade, a number of studies have investigated the role of oxygen in setting thermal limits in aquatic ectotherms (see above), including arthropods. Arthropods represent one of the most species-rich taxonomic groups on Earth. They display a great diversity in the modes of ventilation, circulation and blood oxygen transport, with representatives living both in water (mainly crustaceans) and on land (mainly insects). These two respiratory media have very different consequences for gas exchange (see below), and arthropods therefore represent a suitable group to evaluate support for the OCLTT hypothesis. Although the causal relationships between aerobic scope and growth remain to be established, it is intuitively clear that "excess" capacity for oxygen delivery (MMR > SMR) is required for an animal to allocate aerobic energy to reproduction, growth, and physical activities, including predator avoidance, prey capture, and locomotion. Also, given the prolonged nature of growth and reproduction, these activities are typically supported by aerobic metabolism and hence rely on sufficient aerobic scope (Fig. 1). The idea of "excess" capacity for oxygen delivery is very akin to the capacity of an animal to regulate oxygen uptake (referred to as "*respiratory control*" by Verberk and Atkinson, 2013) and to the concept of oxygen safety margins (e.g., Greenlee and Harrison, 2005). Oxygen safety margins represent the differences between the ambient PO_2 and the critical PO_2 level (P_{crit}) below which oxygen uptake rates can no longer be maintained. A key point here is that arthropod animals differ in their capacity to regulate oxygen uptake and have different oxygen safety margins, which likely reflect selection for increased maximum gas conductance rates through the respiratory system, such as in flying insects or more athletic versus sedentary species (e.g., Reinhold, 1999). Such differences could potentially explain some of the controversy over the generality of the OCLTT hypothesis (Verberk and Bilton, 2013, 2015; Boardman and Terblanche, 2015).

3. Differences between insect and crustacean respiration and between air and water as a respiratory medium

Oxygen can be considered limiting when oxygen delivery does not satisfy metabolic oxygen requirements, leading to an impairment of animal performance. An evaluation of the OCLTT hypothesis must therefore consider the great diversity in the modes of ventilation, circulation, blood oxygen transport and respiratory media among arthropods (Dejours, 1981; Massabuau and Abele, 2012). Most insects breathe air, whereas most crustaceans and many larval insects are aquatic, and it is pertinent therefore to consider the distinct differences between air and water as respiratory media (Dejours, 1981; Hsia et al., 2013; Verberk and Atkinson, 2013). The availability of oxygen is much lower in water compared to air due to the lower diffusion coefficient and solubility of oxygen in water. In contrast to air where temperature exerts a negligible influence on oxygen availability, increased temperature further reduces the solubility of oxygen in water, while maximum rates of oxygen diffusion can be slightly enhanced as the diffusion coefficient of oxygen in water increases with temperature (Dejours, 1981; Verberk et al., 2011). As oxygen consumption increases more than rates of oxygen diffusion, boundary layers become oxygen depleted, and consequently aquatic organism may experience a lower availability of oxygen (Verberk and Atkinson, 2013). Therefore oxygen is more likely to become limiting in water, a problem that may be exacerbated at high temperatures.

Most crustaceans rely on convective oxygen transport by the cardiovascular system. The oxygen-carrying protein hemocyanin augments oxygen transport, and a lowered oxygen affinity of the hemocyanin with increased temperatures may enhance unloading into tissues (Wang and Malte, 2011; Giomi and Pörtner, 2013). In contrast, insects do not typically use a cardiovascular system for gas exchange as the vast majority of the oxygen is conveyed to the metabolizing tissue through the air-filled trachea. Therefore, oxygen transport in insects does not utilize oxygen-carrying proteins (reviewed by Burmester and Hankeln, 2007) and the convective transport of gas can be driven by active contractions of the abdomen to different degrees (see Westneat et al., 2003; Groenewald et al., 2012). Given the higher diffusion rates of oxygen in air, small, terrestrial insects may sustain oxygen delivery to the respiring cells by diffusion alone. Thus, it is clear that the capacity of an animal to deliver oxygen is more readily dependent on diffusive transport in some animals, while convective transport and the concomitant ventilatory and cardiovascular capacities are more important in others (Fig. 2).

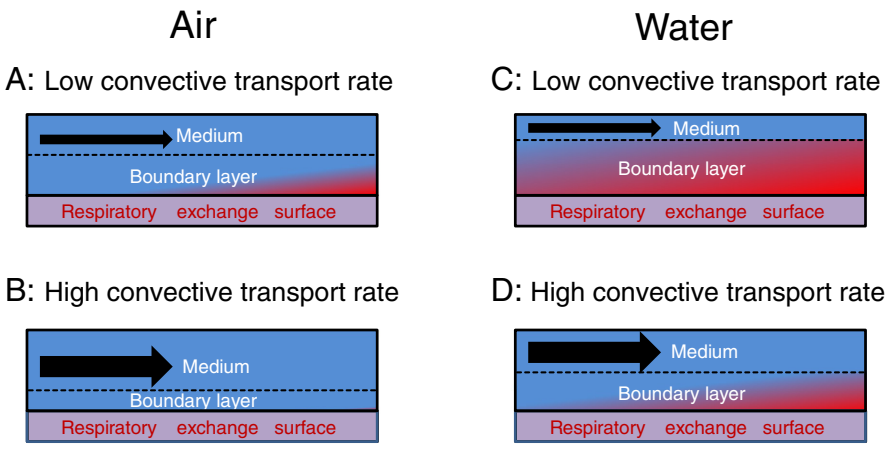

Fig. 2. Schematic illustration of how oxygen uptake depends on the rate of convective transport and how this dependence differs between air and water. In air (A, B), oxygen near respiratory surfaces is less likely to become depleted, even at low convective transport rates. This is because of the higher solubility of oxygen in air than in water and the thinner boundary layer arising from the lower viscosity of air. In addition the much greater diffusivity of oxygen in air allows for a steep gradient from high PO_2 (blue) to low PO_2 (red) to be maintained. In water (C,D), oxygen availability is much more likely to become limiting. As a respiratory media passes over the respiratory surface and oxygen is exchanged the partial pressure of oxygen quickly declines due to the low oxygen carrying capacity. This effect is exacerbated at high temperature because of the higher oxygen exchange rates and due to the decreasing carrying capacity of water at these high temperatures. Only at high convection rates can a steep gradient in PO_2 be maintained and under these conditions, oxygen availability at high temperatures may surpass that at low temperatures, as diffusivity of oxygen increases, and viscosity related effects (e.g., boundary layer thickness) decrease with increasing temperature.

4. Testing the OCLTT hypothesis

The OCLTT hypothesis aims to explain thermal performance curves and the associated pejus temperatures (Fig. 1). Unfortunately, few, and mostly correlative data exist to test the hypothesis within the thermal range set by the upper and lower pejus temperature (Pörtner and Knust, 2007; Eliason et al., 2013; Ferreira et al., 2014). Many studies testing the OCLTT hypothesis have considered tolerance to thermal extremes (T_{crit} and CT_{max}), rather than the more subtle deterioration in long-term performance (e.g., growth and reproduction) which characterize the pejus temperatures. This is understandable, given the difficulty of experimentally establishing causal relationships between oxygen levels and performance curves. However, it has been argued that studying short-term responses to acute heat or cold may be an inappropriate test of the OCLTT (Pörtner, 2014). The OCLTT hypothesis focuses on sustained animal performance and while animals may meet or surpass oxygen demand in the short term, they are not in a steady-state and this could underestimate the importance of mechanisms put forward by the OCLTT hypothesis underpinning thermal performance curves which act in the long term. Thus, it is important to view the capacity for oxygen delivery in relation to requirements for oxygen: together these determine the functional scope for aerobic performance (Bozinovic and Pörtner, 2015). Similarly, Pörtner and Giomi (2013) argue that a mechanistic approach studying short-term responses to acute heating may fail to acknowledge the importance of the evolutionary and ecological context emphasized by the hypothesis. These are valid points, but they cut both ways, with most of the data used to develop the OCLTT hypothesis suffer from the same short-comings, being based primarily on acute temperature changes. While research should strive to emulate reality as much as possible, the recent stress placed on the evolutionary and ecological context makes it difficult for the experimental biologist to rigorously test the OCLTT hypothesis (see also Clark et al., 2013). Here we have formulated three predictions focussing on different aspects of the hypothesis. While tests of each of these predictions cannot prove or disprove the OCLTT hypothesis, as argued above, combined support or absence thereof can indicate whether thermal limits arise from insufficient oxygen delivery in relation to demand and whether this is generally the case in different arthropods.

The first prediction is that anaerobic metabolites accumulate at temperatures close to the critical thermal limit. According to the OCLTT hypothesis, aerobic energy generation becomes insufficient beyond T_{crit}, necessitating the recruitment of anaerobic metabolic pathways. Note that for this prediction, it does not matter whether energy shortage occurs because mitochondria are oxygen limited (as emphasized for heat) or due to mitochondrial failure (as emphasized for cold). In both cases, energy demand outstrips aerobic energy supply necessitating anaerobic metabolism.

The second prediction is that environmental oxygen availability should elevate (hyperoxia) or depress (hypoxia) thermal limits. If lower thermal limits arise from cold-induced mitochondrial failure rather than insufficient oxygen delivery, then the prediction does not work for cold tolerance. In addition, most studies have measured CT_{max} as an empirical endpoint of heat tolerance at which the animal becomes moribund and can no longer escape the adverse temperatures (Lutterschmidt and Hutchison, 1997). CT_{max} may not be equivalent to T_{crit}, the point beyond which aerobic metabolism becomes insufficient to meet total energetic demands and anaerobic metabolism is recruited to avoid energy deficits. However, given that beyond T_{crit}, survival is time limited and fuelled by anaerobic metabolism, the two endpoints should be closely related. The differences between both endpoints (CT_{max} and T_{crit}) depends on the capacity for anaerobic metabolism (i.e., energy store) in relation to the energetic deficit that needs to be covered by anaerobic metabolism (i.e., rates of energy utilization).

The third prediction is that aerobic scope declines in the temperature interval leading up to T_{crit}. Although this seems easy to investigate experimentally, measurements of aerobic scope are not trivial, requiring reliable measurement of both the SMR and the MMR and thus being prone to the error of both components. There is always the concern that SMR is overestimated due to spontaneous activity or digestion and MMR may be underestimated in animals that fail to perform during the maximal exertion tests. Again, the animals may show different responses if measured during steady state conditions or during acute short term exposure. For example, aerobic scope differed between acute and chronic exposure to high temperatures in fish (Norin et al., 2014). This finding is important as the OCLTT hypothesis is primarily concerned with longer thermal challenges emphasizing steady-state conditions, while most tests employ acute thermal challenges. Thus, long-term performance endpoints used as proxies for aerobic transport capacity, such as growth and reproduction may be important for testing the OCLTT hypothesis in an ecological and evolutionary context.

Our aim is to critically evaluate how well these predictions are supported for insects and crustaceans. Furthermore, we assess whether

Table 1
Overview of study results addressing anaerobic metabolism at thermal extremes (either heat or cold). Support for oxygen limitation at thermal extremes is highlighted in orange, while absence of support is highlighted in blue.

Species	Temperature	Oxygen	Support	Metabolites upregulated	Reference
Cold induced anaerobic metabolism					
Insecta					
Coleoptera					
Melasoma collaris	0°C	Anoxia	Yes	Lactate	Meidell (1983)
Diptera					
Belgica antarctica	-10°C	Normoxia	Yes	Alanine, glycerol, succinate	Michaud et al. (2008)
Sacrophaga crassipalpis	4°C	Normoxia	Yes	glycerol, sorbitol, alanine, glutamine, cystathionine, glucose, pyruvate, urea	Michaud and Denlinger (2007)
Lepidoptera					
Ostrinia furnacalis	0–15°C	Anoxia	Yes	Alanine	Goto et al. (2001)
Orthoptera					
Gryllus pennsylvanicus	0°C	Normoxia	No		MacMillan et al. (2012)
Crustacea					
Decapoda					
Maja squinado	-0.3°C	Normoxia	Yes	Succinate	Frederich and Pörtner (2000)
Heat–induced anaerobic metabolism					
Insecta					
Diptera					
Belgica antarctica	30°C	Normoxia	Yes	2–Oxoglutarate/α–ketoglutaric acid	Michaud et al. (2008)
Drosophila melanogaster	36–38°C	Normoxia	Yes	alanine	Malmendal et al. (2006)
Plecoptera					
Dinocras cephalotes nymphs	27–33°C	Hypoxia (5–14%)	Yes	Lactate, glycerol, glycerol–3–phosphate, acetate, succinate, alanine	Verberk et al. (2013)
	35–36°C	Normoxia (20%)	Yes	Glycerol–3–phosphate, succinate	
	37–38°C	Hyperoxia (36–60%)	No		
Crustacea					
Decapoda					
Maja squinado	33.3°C	Normoxia	Yes	Lactate, succinate	Frederich and Pörtner (2000)
Carcinus maenas	>34°C	Normoxia	Yes	Lactate	Jost et al. (2012)
Homarus americanus	30°C	Normoxia	Yes	Lactate	Jost et al. (2012)
Cancer irroratus	28–30°C	Normoxia	Yes	Lactate	Frederich et al. (2009); Jost et al. (2012)
Cladocera					
Dapnia magna	5–35°C	Normoxia	Yes?	Lactate (only in 10 °C acclimated animals)	Paul et al. (2004)

support is different for air and water breathers. Finally, we discuss how support for these predictions (or a lack thereof) has consequences for OCLTT in arthropods and highlight fruitful directions for future research.

4.1. Prediction 1: anaerobic metabolism is recruited at thermal extremes

The studies investigating whether insects and crustaceans use anaerobic metabolism at thermal extremes provide mixed results. There is support for increased anaerobic metabolism close to the upper thermal limits in some species and more support was found for heat tolerance than for cold tolerance (Table 1; Goto et al., 2001; Meidell, 1983). Whether or not anaerobic metabolism is recruited near critical thermal maxima may be endpoint specific. This could explain some of the ambiguity in finding support as studies score different indices of thermal tolerance (e.g., mortality, loss of activity), which may reflect different physiological endpoints and biochemical processes and therefore capture ecological realism to different degrees (e.g., Andersen et al., 2015; reviewed in e.g., Terblanche et al., 2011). Moreover, the presence or absence of some putative anaerobic metabolites, such as alanine, may result from modulation of other biochemical pathways and not necessarily anaerobic metabolism per se (see Bayley et al., 2010 and references therein).

Many insects can enter a reversible cold-induced coma, which constitutes a functional (but not lethal) thermal limit defining the critical thermal minimum CT_{min} (Gibert and Huey, 2001; MacMillan and Sinclair, 2011). However, cold shock/chill coma is seemingly unrelated to elevations in the levels of anaerobic by-products in *Drosophila* (Overgaard et al., 2007) or crickets (MacMillan et al., 2012), while flesh flies show small increases in alanine, but not lactate or succinate (Michaud and Denlinger, 2007). Moreover, ATP levels are typically well defended in firebugs, aphids, crickets and beetles exposed to several days of chill coma (Pullin and Bale, 1988; Kostal et al., 2004; Colinet, 2011; MacMillan et al., 2012). Studies on cold-induced anaerobic metabolism in water breathing arthropods are rare, likely because of the practical problem that water freezes, but there is equivocal evidence for anaerobic metabolism in freeze-tolerant chironomids (Michaud et al., 2008). Within marine crustaceans, the spider crab *Maja squinado* shows elevated succinate levels in the hepatopancreas at −0.3 °C, indicative of anaerobic metabolism, but this was not found in other tissues (Frederich and Pörtner, 2000). Still, oxygen limitation is perhaps most likely to cause anaerobic metabolism in the hepatopancreas, which is a metabolically very active tissue with a high mitochondrial density.

At the upper thermal extreme, several larval and adult arthropods show increased levels of anaerobic metabolites when heat stressed. However, recruitment of anaerobic metabolic pathways cannot be singularly tied to lack of oxygen at the tissue level. For instance, high temperatures may increase membrane permeability and dissipate mitochondrial proton gradients, thus preventing aerobic production of ATP, which may result in the onset of anaerobic metabolism despite sufficient oxygen delivery. For example, Neven et al. (2014) demonstrated that for the air-breathing, tracheated pupae of the codling moth, *Cydia pomonella*, thermal damage to cell membranes and loss of control of oxidation reactions is likely the lethal mechanism at high temperature when oxygen levels are >11 kPa. Thus, it seems that under these conditions, mitochondrial failure sets in first, rather than oxygen delivery capacity being insufficient. This changes under severe hypoxia (oxygen levels < 2 kPa), when oxygen does become limiting at thermal extremes. Moreover, the biochemical versatility among insects (Hoback and Stanley, 2001) can make it difficult to infer recruitment of anaerobic metabolic pathways from changes in putative anaerobic metabolites. Changes in metabolite levels may indicate biochemical end-products produced in preparation of heat stress even when oxygen is plentiful (e.g., Salvucci et al., 1999; Salvucci and Crafts-Brandner, 2000). For example, Malmendal et al. (2006) found increases in whole body levels of alanine in heat-stressed adult *Drosophila melanogaster*, but there was no rise in lactate or succinate, suggesting the mitochondria were employing aerobic metabolism exclusively. Similarly, Mölich et al. (2012) present experimental support that oxygen delivery is not limiting under normoxia at CT_{max} for *D. melanogaster* (see also Lighton, 2007). Thus, for terrestrial insects under normoxic conditions it seems that anaerobic metabolism is not extensively recruited at high stressful temperatures.

The challenges of breathing under water (lower solubility and diffusivity of oxygen, and the higher density and viscosity of water) are likely to render aquatic organisms more prone to oxygen limitation (see paragraph 3). Indeed, there is more support in aquatic ectotherms for increased levels of anaerobic metabolites as animals approach CT_{max} during acute thermal challenges (rates of warming varying between 2 °C h^{-1} to instantaneous). Several anaerobic metabolites were elevated near CT_{max} under normoxia in the aquatic nymphs of the stonefly *Dinocras cephalotes*. Hypoxia further elevated metabolites associated with anaerobic metabolism at high temperatures (and decreased the critical thermal limit), while this signature of anaerobic metabolism was largely abolished under hyperoxia (and critical thermal limits were increased) (Table 1; Verberk et al., 2013). The few aquatic crustaceans that have been investigated also show evidence of accumulation of anaerobic metabolites near CT_{max}. Decapod crabs (*Carcinus maenas* in Jost et al., 2012 and *Cancer irroratus* in Frederich et al., 2009) maintain stable lactate levels in the heart at lower temperatures, but there is a sudden and abrupt rise in lactate concentration at 2–4 °C below CT_{max}. Furthermore, lactate levels remain relatively stable in the heart up until a few degrees below CT_{max} in the Giant freshwater shrimp (*Macrobrachium rosenbergii*) (Ern et al., 2014), whereas the American lobster (*Homarus americanus*) display a gradual rise in lactate with increasing temperature (Jost et al., 2012). Paul et al. (2004) found increased lactate concentrations near CT_{max} in the waterflea *Daphnia magna* when acclimated to 10 °C, but not when acclimated to 20 or 30 °C. Thus, for a number of water breathing arthropods, there is quite strong evidence that anaerobic metabolism is recruited under normoxic conditions at high stressful temperatures.

4.2. Prediction 2: critical thermal limits can be manipulated by changing oxygen availability

To our knowledge, only one published study has addressed how CT_{min} is affected by oxygen availability. Stevens et al. (2010) found no influence of oxygen levels on lower thermal limits in either the terrestrial beetle *Tenebrio molitor* or the isopod *Porcellio scaber* at oxygen tensions between 2 and 40 kPa. A wider range of studies have investigated how hypoxia or hyperoxia affect heat tolerance (CT_{max}) in arthropods (Table 2; Bowler, 1963). In air-breathing arthropods, there is no reduction in CT_{max} during moderate hypoxia (10 kPa), but reductions are reported at severe environmental hypoxia (e.g., <5 kPa) (Table 2). In aquatic arthropods, reductions in CT_{max} under hypoxia appear more pronounced (Table 2). These were mostly insect larval stages with closed trachea, but hypoxia also reduced heat tolerance in adults of *Aphelocheirus aestivalis*, an aquatic hemipteran with open trachea that relies on plastron breathing (Verberk and Bilton, 2013). In the tropical shrimp (*Penaeus monodon*), hypoxia failed to reduce CT_{max}, while in the temperate European crayfish (*Astacus astacus*) hypoxia was associated with a slight reduction in CT_{max} (Ern et al., 2015). Thus, hypoxia can impact heat tolerance, even in air-breathing arthropods, but only when PO_2 levels are sufficiently low (Table 2).

Compared to hypoxia, the effects of hyperoxia on heat tolerance are less studied. The available data suggests that hyperoxia does not improve heat tolerance in air-breathing arthropods (Table 2), and hyperoxia even reduces heat tolerance in some species, perhaps due to the formation of toxic oxygen radicals (see Hetz and Bradley, 2005; Boardman et al., 2012; McCue and De Los Santos, 2013). Hyperoxia was only found to increase CT_{max} in aquatic stonefly nymphs (*Dinocras cephalotes*; Verberk and Bilton, 2013) and in the plastron breathing hemipteran (Verberk and Bilton, 2015). Again, it appears that the prediction that oxygen modulates thermal limits is better supported in aquatic arthropods.

Finding that hypoxia depresses CT_{max} does not in itself imply that oxygen also limits thermal tolerance under normoxia (e.g., Mölich et al., 2012; Neven et al., 2014). Indeed, the ecological relevance of some of these tests depends on whether arthropods do experience such extreme hypoxia during their lives, which seems especially unlikely in terrestrial environments (but see Holter and Spangenberg, 1997; Callier et al., 2015; Pincebourde and Casas, in press). Therefore, elevated CT_{max} under hyperoxia is usually seen to constitute a stronger test of this prediction. From this perspective, it seems that there is very limited support that oxygen delivery is insufficient for the survival of arthropods near thermal extremes. Two nuances can be made, one related to hypoxia and the other to hyperoxia. First, hyperoxia may facilitate oxygen uptake, but if oxygen transport is not likewise increased, this will not result in enhanced oxygen delivery and hyperoxia need not result in increased heat tolerance. Similarly, even if oxygen delivery is enhanced, another process may become critical for an animal's survival (e.g., protein denaturation, or mitochondrial uncoupling). If this next process in line breaks down at a temperature close to the CT_{max} observed at normoxia, there will only be minor effects on CT_{max} observed at hyperoxia. The second nuance is that tests that manipulate oxygen

tensions generally concern animals under resting conditions. Yet increased oxygen requirements beyond standard metabolism (due to activity, feeding, growing etc.), can result in oxygen delivery becoming insufficient even under normoxia. In this sense, short-term responses to acute heating of inactive animals could underestimate the importance of oxygen limitation for animals in their natural environment that have to actively perform, rather than merely staying alive for the duration of the experiment. For example, the dragonfly *Erythemis simplicicollis* was found to be oxygen limited during flight even in normoxia (Harrison and Lighton, 1998). Whether a species is likely to become oxygen limited under normoxia, when animals have to actively perform, could be reflected by a decrease in CT_{max} under hypoxia when animals are inactive. Thus, effects of both hypoxia and hyperoxia on CT_{max} provide information that may be relevant to the OCLTT hypothesis.

From the available evidence, insufficient capacity for oxygen delivery is not likely to limit survival to thermal extremes for most air-breathing species in normoxia. Tracheal oxygen levels in *Attacus atlas* and *Samia cynthia* moth pupae are tightly regulated at 4–5 kPa PO_2 (e.g., Hetz and Bradley, 2005; Terblanche et al., 2008, but see Matthews et al., 2012; Boardman et al., 2012), suggesting that such a low PO_2 already provides sufficient head pressure to meet varying oxygen demands (Massabuau and Abele, 2012). Stronger evidence exists for aquatic species and life stages, where hypoxia is more common and respiration more challenging. Nevertheless, the aquatic nymph of the dragonfly *Cordulegaster boltonii* did not show a reduction in CT_{max} under hypoxia (5 kPa). This species has a highly tracheated branchial chamber and is able to force water convection across this respiratory surface through abdominal movements (Verberk and Bilton, 2013). Similarly, tropical crustaceans do not show reduced aerobic scope until temperatures immediately below the lethal temperatures and their CT_{max} was not reduced in hypoxia (12 kPa) (Ern et al., 2014, 2015). Thus, it is possible also for aquatic arthropods to evolve a high capacity to increase oxygen uptake to meet elevated metabolic demands under warmer conditions (Ern et al., 2015, see also Giomi et al., 2014). A recent comparative study on different orders of insect revealed that hypoxia-induced reductions in CT_{max} co-varied with interspecific differences in capacity to regulate gas exchange (Verberk and Bilton, 2013). Across the four species pairs studied, heat tolerance was consistently less impacted by hypoxia in the species that was better at regulating oxygen uptake. In addition, experimentally reducing the capacity of a bimodal breather to regulate its oxygen uptake, by denying it access to air, induced oxygen-limited heat tolerance (Verberk and Bilton, 2015). Similarly, Boardman and Terblanche (2015) showed that silkmoth larvae, but not pupae are oxygen limited under hypoxia, and related this to their oxygen safety margins, as derived from respirometry at different oxygen levels.

It is therefore perhaps not surprising that the case where hyperoxia increased CT_{max} comes from the aquatic nymphs of the stonefly *Dinocras cephalotes*, which relies on integument and gill breathing (Verberk and Bilton, 2011; Verberk et al., 2013). Also, Whitney (1939) reported increased survival under hyperoxia in aquatic mayfly nymphs exposed for 24 h to stressful temperatures. The degree to which oxygen delivery is constrained is in large part related to the respiratory medium and the capacity to ventilate the respiratory surfaces (Table 2, Fig. 2), with the situation being more dire in water breathing ectotherms. However, there are also examples of air-breathing animals with limited capacity for regulating oxygen uptake. Insect eggs are, for example, likely to have less capacity to regulate oxygen uptake and eggs become oxygen limited under normoxia at high temperatures (Woods and Hill, 2004). Also, moth caterpillars of *Manduca sexta* had reduced oxygen safety margins when nearing molt (Greenlee and Harrison, 2005).

The general picture emerging is that oxygen is not always the limiting factor for acute thermal tolerance under normoxia. It must be noted here that the OCLTT is primarily concerned with longer thermal challenges and whether oxygen is more likely to become limiting during chronic heat stress requires investigating the effects of hypoxia and hyperoxia on performance metrics other than CT_{max} (e.g., see Hoefnagel and Verberk, in press). Yet, oxygen may limit acute heat tolerance in some species, and less so in other species. The extent to which taxa show oxygen limited thermal tolerance may be contingent, depending on a species' capacity to regulate oxygen uptake, which is often tightly linked to their respiratory medium (air vs. water) (Verberk and Bilton, 2013, 2015; Verberk and Atkinson, 2013; Boardman and Terblanche, 2015).

4.3. Prediction 3: aerobic scope and cardiorespiratory performance declines towards thermal limits

Most elements of the oxygen transport cascade as well as resulting SMR, MMR and aerobic scope change with temperature. SMR and MMR, from which aerobic scope was derived, are measured in animals when resting or routinely active, respectively. Several elements of the oxygen transport cascade (e.g., ventilation and cardiac output, and hemolymph oxygen binding properties) have been measured in crustaceans in relation to the OCLTT hypothesis, whereas similar measurements are sorely lacking for insects. One study on an insect (Mormon cricket, *Anabrus simplex*; Chappell et al., 2009) showed that relative aerobic scope fell with increasing temperature, while absolute aerobic scope plateaued. However, given the scarcity of data on insects, we primarily discuss the crustacean literature in relation to the oxygen transport cascade and OCLTT.

According to the OCLTT hypothesis, aerobic scope is typically presented as a curve with a maximum value close to the preferred body temperature and progressive declines in scope as temperature approaches upper or lower T_{crit} (Fig. 1). These reductions in scope are ascribed to progressive failure of the cardiorespiratory systems, and the OCLTT hypothesis therefore implies that as temperatures deviate from T_{opt}, heart rates, gill ventilation rates, as well hemolymph oxygen levels are reduced. Such data were presented in the pioneering study by Frederich and Pörtner (2000) on spider crab *Maja squinado* showing accompanying accumulation of anaerobic metabolites as temperatures approached T_{crit}. Similar findings were subsequently reported for various crustaceans providing additional support for the OCLTT hypothesis (e.g., Storch et al., 2009; Storch et al., 2011; Frederich et al., 2009; see Table 3). However, several more recent studies provide examples of species where aerobic scope only starts to decrease a few degrees Celcius below the lethal temperature. Since it is inevitable that aerobic scope falls as the animal succumbs to critical temperatures, such data has been interpreted as being in opposition to the OCLTT hypothesis. However, it is not straightforward to define the exact temperature difference between T_{crit} and temperature where aerobic scope decreases (T_{pejus}). Similarly, it is not easy to define how much elements of the oxygen transport cascade should decrease before the data is interpreted as support for OCLTT, although the OCLTT theory predicts a stagnation and decline in Q_{10} values within the high temperature range. Therefore, for the purpose of the present review, we use Q_{10} values that are below 1.2 as indications that elements of the oxygen transport cascade are impaired (as the Q_{10} value of tissue oxygen requirement is likely to be above 1.2). Lower Q_{10} values are therefore taken as indicative of progressive reduction in oxygen delivery capacity.

Heart and ventilation rates have been used as measures to evaluate the thermal dependence of the oxygen uptake and transport systems and therefore also OCLTT, with the prediction of cardiorespiratory failure as temperatures approach T_{crit}. Heart rate has been reported in several studies, probably because it is relatively easy to measure with minimal disturbance of the experimental animal. Although heart rate alone does not reveal cardiac output as it also depends on stroke volume, its stagnation and decrease has been used as an indication of loss of performance (Frederich and Pörtner, 2000). There is, however, very little evidence for stagnation ($1.2 > Q_{10} > 0.8$) or decline ($Q_{10} < 0.8$) in heart rate within the upper pejus range (Table 3, Fig. 3). This is perhaps unsurprising

Table 2
Overview of study results addressing the effect of oxygen availability on maximum critical temperatures. Support for oxygen limitation at thermal extremes is highlighted in orange, while absence of support is highlighted in blue. Not measured (n.m.) indicate the prediction was not measured in the study.

Lifestage, species	Respiration[a]	Hypoxia lowers upper thermal tolerance	Hyperoxia improves upper thermal tolerance	Method[b]	Reference
Insecta					
Blattodea					
Adult *Gromphadorhina portentosa*	cA	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Gromphadorhina portentosa nymphs	cA/dA1	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Coleoptera					
Adult *Agabus bipustulatus*	cA	5 kPa	n.m.	CT	Verberk and Bilton (2013)
Adult *Gonocephalum simplex*	cA	2.5 kPa	40 kPa	CT (TLR)	Klok et al. (2004)
Adult *Tenebrio molitor*	cA	2.5 kPa	40 kPa	CT (TLR)	Stevens et al. (2010)
Adult *Tenebrio molitor*	cA	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Tenebrio molitor larvae	cA	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Adult *Zophobus morio*	cA	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Adult *Hippodamia convergens*	cA	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Adult *Limnius volckmari*	dW2	5 kPa	n.m.	CT	Verberk and Bilton (2013)
Diptera					
Adult *Drosophila melanogaster*	cA/dA1?	<10 kPa	n.m.	CT (ADS)	Lighton (2007)
Ephemeroptera					
Ecdyonurus insignis nymphs	cW1	5 kPa	n.m.	CT	Verberk and Bilton (2013)
Rhitrogena semicolorata nymphs	dW1	5 kPa	n.m.	CT	Verberk and Bilton (2013)
Hemiptera					
Adult *Ilyocorus cimicoides*	cA + dW2	5 kPa	60 kPa	CT	Verberk and Bilton (2013); (2015)
	dW2	5 kPa	60 kPa	CT	Verberk and Bilton; (2015)
Adult *Aphelocheirus aestivalis*	dW2	5 kPa	60 kPa	CT	Verberk and Bilton (2013); 2015
Hymenoptera					
Adult *Pogonomyrmex occidentalis*	cA/dA1?	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Lepidoptera					
Bombyx mori larvae	cA	2.5 kPa	40 kPa	CT (TLR)	Boardman and Terblanche (2015)
Bombyx mori pupae	cA	2.5 kPa	n.m.	CT (TLR)	Boardman and Terblanche (2015)
Odonata					
Cordulegaster boltonii nymphs	cW2	5 kPa	n.m.	CT	Verberk and Bilton (2013)
Calopteryx virgo nymphs	dW1	5 kPa	60 kPa	CT	Verberk and Calosi (2012); Verberk and Bilton (2013)
Orthoptera					
Adult *Acheta domesticus*	cA	10 kPa	35 & 95 kPa	LT_{50}	McCue and De Los Santos (2013)
Plecoptera					
Dinocras cephalotes nymphs	dW1	5 & 14 kPa	36 & 60 kPa	CT	Verberk et al. (2013); Verberk and Bilton (2011)
Crustacea					
Decapoda					
Adult *Penaeus monodon*	cW2	12 kPa	n.m.	CT	Ern et al. (2015)
Adult *Astacus astacus*	cW2	12 kPa	n.m.	CT	Ern et al. (2015)
Adult *Austropotamobius pallipes*	cW2	n.m.	Close to 100 kPa	LT_{50}	Bowler (1963)
Isopoda					
Adult *Porcellio scaber*	dA2	2.5 kPa	40 kPa	CT (TLR)	Stevens et al. (2010)
Adult *Armadillidium vulgare*	dA2	2.5 kPa	40 kPa	CT (TLR)	Klok et al. (2004)

given that the crustacean heart is placed downstream of the gills in most crustaceans, making oxygen shortage in the cardiac tissue unlikely (Ern et al., 2014). This contrasts with fish, where the heart is placed just upstream of the gills, making the fish heart more prone to hypoxemia (Wang and Overgaard, 2007). Although studied in less detail than heart rate, most studies actually report increases in ventilation rates up to the critical temperature range with $Q_{10} > 1.2$ (Wittmann et al., 2012; Giomi and Pörtner, 2013; Ern et al., 2014, 2015) (Table 3, Fig. 3). However, the thermal increase in ventilation rates within the upper pejus range was usually much reduced compared to the Q_{10} values observed at lower temperatures (Table 3), suggestive of breakpoints. The same pattern was observed for oxygen consumption rates, while for heart rates Q_{10} values did not seem to exhibit such breakpoints. Together this suggests that ventilation may more readily become limiting in aquatic crustaceans under warm water conditions (see Fig. 2).

Support for the OCLTT hypothesis in crustaceans is given by declining oxygen partial pressures measured in the pericardial sinus when temperature increases towards T_{crit} in crustacea ($Q_{10} < 0.8$; Metzger et al., 2007; Frederich and Pörtner, 2000; Walther et al., 2009; Giomi and Pörtner, 2013; Wittmann et al., 2012) (Table 3, Fig. 3). The decline in arterial PO_2 is indicative of the respiratory surfaces being unable to saturate the hemocyanin that, in combination with the lowered hemolymph oxygen affinity, is predicted to lower arterial oxygen concentration. The reduced arterial oxygen levels could themselves be responsible for the concomitant lowering of venous oxygen levels, but reduced cardiac output relative to oxygen uptake could further exacerbate the decline in venous oxygen levels. To disentangle whether the observed lowering of arterial oxygen levels relates to temperature enhanced oxygen consumption or reduced cardiac output, it would be interesting to repeat these studies in animals with increased metabolism, whether due to exercise or digestion. Giomi and Pörtner (2013) recently measured components of the oxygen transport cascade in the common green crab (*Carcinus maenas*) as temperature rose and argued that the presence of hemocyanin widened the thermal tolerance window, with model considerations indicating that hemocyanin extends T_{crit} from 16 to 22 °C for crabs acclimated to 10 °C. Nevertheless, McGaw and Whiteley (2012) showed that *C. maenas*, similarly acclimated to 10 °C could still increase oxygen uptake during digestion (specific dynamic action), demonstrating a factorial aerobic scope of almost 1.5 at 25 °C. Thus, the two studies highlight that inferring critical temperatures based on extrapolated blood parameters compared to measured aerobic scope can lead to different conclusions regarding T_{crit}. Alternatively, the two experimental populations could have differed in their physiology.

Very few studies have directly estimated the aerobic scope in crustaceans and those that have do not show strong declines in aerobic scope (Q_{10} ranging between 0.9 to 1.1) and thus fail to support the prediction of aerobic scope declining towards zero at T_{crit}. Most studies reporting oxygen consumption across a range of temperatures lack rigorous measurements of SMR and MMR. Reported oxygen consumption values thus frequently represent the less well-defined routine metabolism, which is a mix of SMR with an undetermined component of activity or digestion (Withers, 1992). Reduced routine metabolism towards critical temperatures therefore represents a combination of an increased SMR reflecting elevated maintenance costs, combined with either a loss of oxygen delivery capacity or decreased activity during measurements, or a combination of both (see also Halcrow and Boyd, 1967). This makes it difficult to draw unequivocal conclusions from data on routine metabolism alone. Routine metabolism was found to increase within the upper pejus range, suggesting that MMR does not plateau or decline (Fig. 3). Whether animals are indeed oxygen-limited near critical temperatures or whether they still have some residual aerobic scope cannot be concluded from such data as it depends on the thermal dependency of SMR. For *Macrobrachium rosenbergii*, a tropical freshwater crustacean, maximum oxygen uptake showed no evidence of failing oxygen deliver close to T_{crit}, and 76% of its aerobic scope measured at T_{opt} remained close to T_{crit} (Ern et al., 2014).

5. Discussion

Although based on much earlier publications, the OCLTT hypothesis has been enthusiastically received over the past decade, motivating a diverse spectrum of studies from ecology and behavior, to classic physiological investigations of the mechanisms dictating organismal function. Much of the intuitive appeal of the OCLTT hypothesis probably resides with the proposed linkage of a relatively simple physiological mechanism, the oxygen uptake and transport systems, to a very complicated and often frustratingly difficult interplay between abiotic and biotic influences on fitness, growth and reproduction of animals in their native environment. Such integrative linkage at many levels of biological organization, i.e., from whole-organism fitness to mitochondrial ATP synthesis, is a desirable academic goal. Nevertheless, much controversy remains as to whether the OCLTT hypothesis can be regarded as a unifying principle but both skeptics and supporters will agree that the OCLTT hypothesis must be thoroughly and critically evaluated. Such scrutiny should preferably involve experimental manipulation where oxygen delivery capacity or oxygen availability is altered to unravel the factors that influence and dictate thermal tolerance in a variety of animals with diverse modes of respiration.

Based on the available literature, we conclude that there is mixed support for the predictions derived from the OCLTT hypothesis in arthropods. The relatively few studies investigating the OCLTT hypothesis at low temperature do not support a universal role for oxygen in setting the lower thermal limits in arthropods. For example, there is no evidence that cold tolerance is modulated by ambient oxygen levels (Stevens et al., 2010) and accompanied by decreased energy status (drop in ATP levels), while elevated anaerobic metabolites were only reported in the hepatopancreas of a temperate crustacean (Frederich and Pörtner, 2000). While aerobic scope and oxygen delivery capacity may decline at low temperatures, these reductions do not appear to influence cold tolerance per se in arthropods. More data is certainly available with respect to heat tolerance, and several predictions derived from the OCLTT hypothesis find some support in the experimental evidence. Overall, support for our three predictions seems to be strongest for increased anaerobic metabolism close to CT_{max} (Table 1). In contrast, mixed support was found for the prediction of decreased aerobic scope near critical temperatures, a key feature of the OCLTT hypothesis. Finally, CT_{max} of several arthropods is affected by manipulation of oxygen tension (Table 2), with strongest support for this prediction being found in aquatic arthropods. Verberk and Bilton (2013) demonstrated that these discrepancies between water and air breathing arthropods can be largely reconciled by taking the differences in capacity to

Notes to Table 2

[a] Respiration type, which is dependent on convection or diffusion in either air or water (see also Fig. 3): cA: convection of air into open trachea and then directly to metabolizing tissue; dA1: diffusion of air into metabolizing tissue (via open trachea or not); dA2: diffusion of air into hemolymph where convection via a cardiovascular system delivers oxygen to metabolizing tissue; cW1: convection of water to aid diffusion of dissolved oxygen into closed trachea from which it diffuses into metabolizing tissue; cW2: convection of water to aid diffusion of dissolved oxygen into hemolymph where convection via a cardiovascular system delivers oxygen to metabolizing tissue; dW1: diffusion of dissolved oxygen into closed trachea from which it diffuses into metabolizing tissue; dW2: diffusion of dissolved oxygen into an air store (plastron/physical gill) from which convection of air takes place into open trachea and then directly to metabolizing tissue.

[b] Method used to establish critical temperatures: CT: temperature is increased at a constant rate until the endpoint is reached, at which point the animal loses its ability to escape from the surrounding conditions that will promptly lead to its death (Lutterschmidt and Hutchison, 1997). This endpoint can be established by thermolimit respirometry (TLR, where a CO_2 post mortem peak or cessation of spiracle activity is measured), or by the cessation of activity (ADS). LT_{50}: exposure to a constant temperature and recording the time until death.

Table 3
Published studies on the effects of temperature on oxygen supply capacity parameters in crustaceans, including heart rate, gill ventilation rate, routine metabolic rate (RMR), absolute aerobic scope and arterial and venous hemolymph oxygen tension. Since there is a large variation in the temperature range of studied animals, the fraction of that range that is reported and the extent of change observed, we have normalized the published data by dividing each studies temperature interval into 6 equal zones (representing lower critical, lower pejus, lower optimal, upper optimal, upper pejus and upper critical; Pörtner, 2002, 2010; Pörtner, 2012) and calculated the Q_{10} for each temperature interval and each included parameter. In studies where trend lines have been fitted to the measured values these line values have been used instead of the individual data points. In studies where the effects of temperature have been measured before and after experimental treatments such as hypercapnia or hypoxia exposure only control values have been used. Not measured (n.m.) indicate the parameter was not measured at this temperature interval. This meta-analysis is summarized in Fig. 3 for the reported parameters, where the measured parameter in the upper pejus temperature intervals (highlighted in gray) has then been categorized as either increasing ($Q_{10} > 1.2$), showing equivocal change ($1.2 > Q_{10} < 0.8$) or decreasing ($Q_{10} < 0.8$).

Species (Common name)	Temperature range (°C)	Temperature change	Heart rate (beats min^{-1})		Gill ventilation rate (beats min^{-1})		Oxygen uptake				Hemolymph oxygen tension		Reference
			Range	Q_{10}	Range	Q_{10}	RMR	Q_{10}	Absolute Scope	Q_{10}	PO_2	Q_{10}	
Astacus astacus (European crayfish)	18–21.5	Acute: 2 °C h^{-1}	87–106	1.7	62–63	1.1	22–27	1.7	69–86	–			Ern et al. (2015)
	21.5–25		106–125	1.6	63–89	2.7	27–31	1.5	86–95	–			
	25–28.5		125–156	1.9	89–145	4.0	31–42	2.3	95–93				
	28.5–32		156–203	2.1	145–238	4.2	42–54	2.1	93–82				
	32–35.5		203–206	1.1	238–126	0.2	n.m.	–	n.m.				
	35.5–38		206–0	–	126–0	–	n.m.	–	n.m.				
Cancer irroratus (Rock crab)	11–15	Acute: 6 °C h^{-1}	50–65	1.9									Frederich et al. (2009)
	15–18		65–95	3.5									
	18–22		95–105	1.3									
	22–25		105–150	3.3									
	25–29		150–145	0.9									
	29–32		145–45	–									
Cancer pagurus (Edible crab)	10–12	Acute: 1 °C h^{-1}									11–9	0.4	Metzger et al. (2007)
	12–14										9–9	1.0	
	14–16										9–7.5	0.4	
	16–18										7.5–4	0.04	
	18–20										4–2	0.03	
	20–22										2–1	0.03	
Carcinus maenas (Green crab)	5–9	Instantaneous	34–44	1.9									Ahsanullah and Newell (1971)
	9–13		44–53	1.6									
	13–17		53–66	1.7									
	17–21		66–84	1.8									
	21–25		84–95	1.4									
	25–30		95–66	0.5									
Carcinus maenas (Green crab)	5–9	Chronic: 14–21 days	34–44	1.9									Ahsanullah and Newell (1971)
	9–12		44–56	2.2									
	12–15		56–76	2.8									
	15–18		76–84	1.4									
	18–21		84–93	1.4									
	21–25		93–104	1.3									
Carcinus maenas (Green crab)	7–10	Chronic: >4 weeks					14.6–22.1	4.0	17.0–36.1	12.3			Robertson et al. (2002)
	10–13						22.1–29.7	2.7	36.1–55.4	4.2			
	13–15						29.7–34.7	2.2	55.4–68.0	2.8			
	15–18						34.7–44.9	2.4	68.0–70.2	1.1			
	18–20						44.9–51.8	2.0	70.2–71.9	1.1			
	20–22						51.8–58.6	1.9	71.9–73.4	1.1			
Carcinus maenas (Green crab)	5–8	Acute: 15 °C h^{-1} (*Acclimated*, 10 °C)					10–21	11.9	19–35	7.4			McGaw and Whiteley (2012)
	8–11						21–31	3.7	35–42	1.9			
	11–15						31–44	2.4	42–44	1.1			
	15–18						44–64	3.5	44–54	2.0			
	18–21						64–84	2.5	54–55	1.0			
	21–25						84–110	2.0	55–44	0.6			
Carcinus maenas (Green crab)	5–8	Acute: 15 °C h^{-1} (*Acclimated*, 20 °C)					8–13	5.0	25–40	4.5			McGaw and Whiteley (2012)
	8–11						13–19	3.5	40–56	3.2			
	11–15						19–27	2.4	56–76	2.1			
	15–18						27–48	6.8	76–106	3.0			
	18–21						48–69	3.4	106–110	1.1			
	21–25						69–98	2.4	110–88	0.6			
Carcinus maenas (Green crab)	11–17	Acute: 6 °C h^{-1}	45–57	1.5									Jost et al. (2012)
	17–20		57–85	3.8									
	20–26		85–135	2.2									
	26–29		135–160	1.8									
	29–32		160–200	2.1									
	32–35		200–175	0.6									
Carcinus maenas (Green crab)	10–12	Acute: 1 °C h^{-1}	62–69	1.7	6.5–6	0.7	22.5–31	5.0			140–115	0.4	Giomi and Pörtner (2013)
	12–14		69–76	1.6	6–6.5	1.5	31–40	3.6			115–100	0.5	
	14–16		76–82	1.5	6.5–9.5	6.7	40–49	2.8			100–80	0.3	
	16–19		82–86	1.2	9.5–13.5	3.2	49–58	1.8			80–65	0.5	
	19–22		86–91	1.2	13.5–18	2.6	58–63.5	1.4			65–55	0.6	
	22–25												

(continued on next page)

Table 3 (*continued*)

Species (Common name)	Temperature range (°C)	Temperature change	Heart rate (beats min^{-1})		Gill ventilation rate (beats min^{-1})		Oxygen uptake				Hemolymph oxygen tension		Reference
			Range	Q_{10}	Range	Q_{10}	RMR	Q_{10}	Absolute Scope	Q_{10}	PO_2	Q_{10}	
			91–95	1.2	18–23.5	2.4	63.5–68.5	1.3			55–45	0.5	
Homarus americanus (Hard shell lobsters)	14–18 18–21 21–24 24–27 27–30 30–33	Acute: 6 °C h^{-1}	50–60 60–70 70–100 100–110 110–140 140–5	1.6 1.7 3.3 1.4 2.2 –									Jost et al. (2012)
Hyas araneus (Great spider crab)	0–4 4–8 8–12 12–16 16–20 20–25	Acute: 1 °C h^{-1}	0–4 4–8 8–12 12–16 16–20 20–25	1.2 1.2 1.5 1.0 1.0 1.0							12.6–11.6 11.6–10.4 10.4–7.2 7.2–4.8 4.8–3.4 3.4–2.4	0.8 0.8 0.4 0.4 0.4 0.5	Walther et al. (2009)
Hyas araneus (Spider crab, zoea I)	10–13 13–16 16–19 19–22 22–25 25–28	Acute: 6 °C h^{-1}	190–223 223–255 255–270 270–335 335–415 415–117	1.7 1.6 1.2 2.1 2.0 0.1	320–275 275–335 335–255 255–253 253–235 235–1	0.6 1.9 0.4 1.0 0.8 0.1	1.14–1.46 1.46–1.59 1.59–2.00 2.00–2.20 2.20–2.35 2.35–0.27	2.3 1.3 2.1 1.4 1.2 0.1					Schiffer et al. (2014)
Hyas araneus (Spider crab, zoea II)	10–13 13–16 16–19 19–22 22–25 25–28	Acute: 6 °C h^{-1}	147–173 173–217 217–245 245–327 327–393 393–153	1.7 2.1 1.5 2.6 1.8 0.1	410–397 397–390 390–335 335–215 215–145 145–1	0.9 0.9 0.6 0.2 0.3 0.1	1.00–1.26 1.26–1.42 1.42–1.95 1.95–2.07 2.07–2.27 2.27–0.16	2.2 1.5 2.9 1.2 1.4 0.1					Schiffer et al. (2014)
Hyas araneus (Spider crab, megalopa larvae)	10–13 13–16 16–19 19–22 22–25 25–28	Acute: 6 °C h^{-1}	155–170 170–240 240–235 235–240 240–81 81–0	1.4 3.2 0.9 1.1 0.1 0.1			0.29–1.05 1.05–1.56 1.56–1.53 1.53–1.99 1.99–0.55 0.55–0.05	72.9 3.7 0.9 2.4 0.1 0.1					Schiffer et al. (2014)
Macrobrachium rosenbergii (Giant freshwater shrimp)	30–32 32–34 34–36 36–38 38–40 40–42	Acute: 2 °C h^{-1}	171–203 203–242 242–277 277–317 317–358 358–0	2.4 2.4 2.0 2.0 1.8 –	116–117 117–167 167–288 288–455 455–519 519–0	1.0 5.9 15.3 9.8 1.9 –	179–208 208–241 241–289 289–344 n.m. n.m	2.2 2.1 2.5 2.4 – –	253–232 232–217 217–198 198–193 n.m. n.m.	0.6 0.7 0.6 0.9 – –			Ern et al. (2014)
Maja squinado (European spider crab)	0–10 10–20 20–25 25–30 30–35 35–40	Acute: 1–2 °C h^{-1}	5–46 46–74 74–74 74–80 104–60 60–2	9.2 1.6 1.0 1.2 0.3 –	1–34 34–100 100–84 84–86 86–28 28–1	34.0 2.9 0.7 1.1 0.1 –					8–92 92–72 72–28 28–20 20–8 8–8	11.5 0.8 0.2 0.5 0.2 1.0	Frederich and Pörtner (2000)
Paralomis granulosa (Stone Crab)	−1–1 1–4 4–7 7–9 9–11 11–13	Acute: 1 °C h^{-1}	13–16 16–17 17–22 22–29 29–34 34–44	2.8 1.2 2.4 4.0 2.2 3.6	23–26 26–30 30–35 35–37 37–40 40–45	1.8 1.6 1.7 1.3 1.5 1.8					14.5–15 15–11.5 11.5–6.0 6–4.5.0 4.5–4.0 4–3.5.0	1.2 0.4 0.1 0.2 0.6 0.5	Wittmann et al. (2012)
Penaeus monodon (Giant tiger shrimp)	30–32 32–34 34–36 36–38 38–40 40–42	Acute: 2–4 °C h^{-1}	106–118 118–164 164–210 210–277 277–306 306–0	1.7 5.2 3.4 4.1 1.6 –	86–155 155–165 165–242 242–370 370–412 412–0	18.7 1.4 6.9 8.2 1.7 –	77–100 100–133 133–164 164–206 n.m. n.m.	3.8 4.1 2.9 3.1 – –	266–273 273–261 261–242 242–207 n.m. n.m.	1.1 0.8 0.7 0.5 – –			Ern et al. (2015)
Petrolisthes cinctipes (Porcelain crab)	1–7 7–12 12–18 18–23 23–29 29–34	Acute: 4 °C h^{-1}	20–110 110–148 148–221 221–270 270–330 330–0	17.1 1.8 1.9 1.5 1.4 –									Stillman and Somero (1996)
Petrolisthes eriomerus (Porcelain crab)	2–6 6–10 10–14 14–18 18–23 23–27	Acute: 4 °C h^{-1}	0–95 95–135 135–181 181–221 221–270 270–0	– 2.4 2.1 1.6 1.5 –									Stillman and Somero (1996)
Sagmariasus verreauxi (Spiny lobsters, puerulus)	15–17.5 17.5–20	Acclimated, 2–3 days					0.33–0.35 0.35–0.40	1.3 1.7	0.69–0.94 0.94–1.13	3.4 2.1			Fitzgibbon et al. (2014)

Table 3 (*continued*)

Species (Common name)	Temperature range (°C)	Temperature change	Heart rate (beats min^{-1})		Gill ventilation rate (beats min^{-1})		Oxygen uptake				Hemolymph oxygen tension		Reference
			Range	Q_{10}	Range	Q_{10}	RMR	Q_{10}	Absolute Scope	Q_{10}	PO_2	Q_{10}	
Sagmariasus verreauxi (Spiny lobsters, puerulus)	20–22.5						0.40–0.47	1.9	1.13–1.24	1.5			
	22.5–25						0.47–0.55	1.9	1.24–1.26	1.1			
	25–27.5						0.55–0.63	1.7	1.26–1.22	0.9			
	27.5–30						0.63–0.64	1.1	1.22–1.05	0.5			
Taliepus dentatus (Kelp crab, Zoea I) Southern Chile	3–7	Acute: 2 °C h^{-1}	120–180	2.8			0.31–0.65	6.4					Storch et al. (2009)
	7–11		180–220	1.7			0.65–0.56	0.7					
	11–15		220–280	1.8			0.56–0.80	2.4					
	15–19		280–385	2.2			0.80–1.06	2.0					
	19–23		385–475	1.7			1.06–1.06	1.0					
	23–27		475–440	0.8			1.06–0.72	0.4					
Taliepus dentatus (Kelp crab, Zoea I) Central Chile	3–7	Acute: 2 °C h^{-1}	90–185	6.1			0.22–0.40	4.5					Storch et al. (2009)
	7–11		185–210	1.4			0.40–0.38	0.9					
	11–15		210–275	2.0			0.38–0.62	3.4					
	15–19		275–375	2.2			0.62–0.82	2.0					
	19–23		375–465	1.7			0.82–0.85	1.1					
	23–27		465–470	1.0			0.85–0.70	0.6					
Taliepus dentatus (Kelp crab, Zoea I)	3–7	Acute: 2 °C h^{-1}	90–175	5.3			0.13–0.46	23.6					Storch et al. (2011)
	7–11		175–185	1.2			0.46–0.29	0.3					
	11–15		185–255	2.2			0.29–0.49	3.7					
	15–19		255–335	2.0			0.49–0.70	2.4					
	19–23		335–445	2.0			0.70–1.02	2.6					
	23–27		445–455	1.1			1.02–1.09	1.2					
Taliepus dentatus (Kelp crab, Zoea II)	3–7	Acute: 2 °C h^{-1}	75–180	8.9			0.10–0.53	64.7					Storch et al. (2011)
	7–11		180–250	2.3			0.53–0.67	1.8					
	11–15		250–315	1.8			0.67–0.67	1.0					
	15–19		315–425	2.1			0.67–0.84	1.8					
	19–23		425–520	1.7			0.84–0.75	0.8					
	23–27		520–265	0.2			0.75–0.23	–					
Taliepus dentatus (Kelp crab, Megalopa)	3–7	Acute: 2 °C h^{-1}	95–180	4.9			0.04–0.26	108					Storch et al. (2011)
	7–11		180–260	2.5			0.26–0.43	3.5					
	11–15		260–330	1.8			0.43–0.50	1.5					
	15–19		330–385	1.5			0.50–0.64	1.8					
	19–23		385–495	1.9			0.64–0.37	0.3					
	23–27		495–555	1.3			0.37–0.18	0.2					

regulate oxygen uptake into account (degree of convective vs. diffusive transport and breathing medium, see Fig. 2). As differences in the

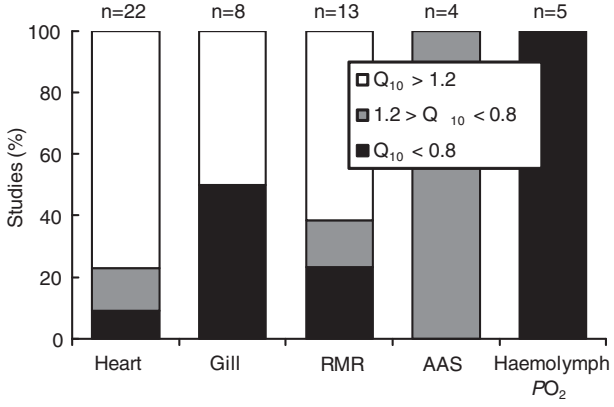

Fig. 3. Summary of the effects of temperature on oxygen uptake and transport parameters in crustaceans, including heart rate, gill ventilation rate, routine metabolic rate (RMR), absolute aerobic scope (AAS) and hemolymph oxygen tension (PO_2) as proxy for the mismatch between oxygen supply and demand (see Table 3). Studies are categorized according to the thermal sensitivity (expressed in Q_{10} values) reported in the temperature zone corresponding to the upper pejus range (see text for details). To make a conservative evaluation of potential mismatches between oxygen requirements and oxygen delivery, we assume that thermal dependency of tissue oxygen requirement is likely to have a Q_{10} value above 1.2, and therefore Q_{10} values for heart rate, gill ventilation rate, RMR and hemolymph PO_2 that are below 1.2 can be taken as indications that elements of the oxygen transport cascade are impaired. Such impairment is also indicated by Q_{10} values for AAS below 0.8 (criteria are more stringent here as AAS already relates maximum oxygen uptake to SMR).

capacity for oxygen delivery alter the extent of oxygen limitation in a predictable manner, the immediate conclusion is therefore that OCLTT does not seem to be a unifying principle to understand tolerance of thermal extremes, as air breathing arthropods are unlikely to be oxygen limited under normoxia. Instead, we support the notion that oxygen limitation is one of several important physiological problems that ectotherms may face at high temperatures (Schmidt-Nielsen, 1997), being more relevant for water breathing arthropods than for air-breathers. Clearly oxygen limitation is more likely to become a problem for organismal performance under hypoxic conditions and temperature-dependent tolerance of hypoxic conditions could successfully predict species distribution patterns (Deutsch et al., 2015). Negative effects of hypoxia are manifested faster and more severely at high temperatures and while this is *concordant* with the OCLTT hypothesis, it is important to realize that this fact by itself does not provide support for heat tolerance being oxygen-limited under normoxia. Nevertheless, many animals may simultaneously face hypoxia and thermal extremes and the combination of these potential stressors is therefore highly relevant, particularly for aquatic organisms where hypoxia (and hyperoxia) is more likely to occur. Future research may reveal that OCLTT could have different degrees of relevance at different life stages (Storch et al., 2009; Boardman and Terblanche, 2015), especially if these have different gas exchange mechanisms giving them different capacities to increase oxygen uptake (Verberk and Bilton, 2015).

6. Future perspectives

Given the attractiveness of the OCLTT hypothesis and the vast number of recent publications reporting on the temperature effects on

metabolism and aerobic scope, surprisingly few studies tested multiple predictions in the same organism, with most studies typically addressing a single key prediction. It is also apparent that most insect studies have addressed either the presence/absence of anaerobic metabolites or how thermal limits are affected by altered oxygen availability in the environment, whereas most studies of crustaceans have focussed on aspects of the oxygen transport pathways (cardiorespiratory parameters) as well as oxygen consumption and aerobic scope. It would be fruitful to have insect studies on aerobic scope and more crustacean studies on tolerance to thermal extremes and especially to have future studies that address multiple predictions in the same species. Information on a larger set of species could highlight whether specific evolutionary modifications modulate the relevance of OCLTT such as their mode of gas exchange (see e.g., Verberk and Bilton, 2013; Giomi et al., 2014).

Another knowledge-gap is the timescale over which responses have been measured. Here we have reviewed studies investigating acute lethal responses, mainly because data on long-term performance remains scarce. Since the OCLTT makes predictions on performance, with implicit predictions on tolerance to extreme temperature, we can only test predictions derived from the OCLTT hypothesis, rather than falsify or confirm the hypothesis itself. Some of these predictions form key elements used to develop the hypothesis in the first place (anaerobic metabolites, aerobic scope, heart rate), although the hypothesis emphasizes sublethal thermal limits beyond which performance declines as a result of the mismatch between oxygen delivery and oxygen utilization (Giomi and Pörtner, 2013; Pörtner, 2014). More rigorous tests of the OCLTT hypothesis need to focus on sub-lethal limits and thermal limitation of performance. Sublethal limits generally involve less extreme temperatures and longer time scales, providing opportunities to investigate the link between aerobic scope and optimal temperatures for physical activity, digestive efficiency, growth and reproduction. Longer timescales would also address a serious concern regarding the rates of heating and cooling employed in most experimental studies reporting effects of acute heating. These rates rarely resemble those occurring in natural environments, and certainly never mimic those relevant for global warming where even the most gloomy forecasts predict less than a degree Celsius rise per decade (IPCC, 2013). Given the experimental difficulties associated with these long-term studies, an intermediate position could be to investigate studies employing prolonged thermal acclimation. Insects and crustaceans adjust CT_{max} after pre-exposure to higher temperatures (Lagerspetz and Bowler, 1989; Calosi et al., 2010) and hypoxia (Verberk and Calosi, 2012). However, linking these acclimatory changes in thermal tolerance to changes in oxygen delivery capacity would be informative (see e.g., Paul et al., 2004). Similarly, changes in hypoxia tolerance under different temperatures can be informative (see Deutsch et al., 2015). Together such studies can shed light on the generality of the OCLTT hypothesis across insects and crustaceans.

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